TRIPEPTIDE COMPOUNDS USEFUL AS SELECTIVE INHIBITORS OF AMINOPEPTIDASE A AND CORRESPONDING PHARMACEUTICAL COMPOSITIONS

The present invention relates to new compounds of therapeutic interest which may be used particularly as selective inhibitors of aminopeptidase A and the corresponding pharmaceutical compositions.

Aminopeptidase A is a zinc ectopeptidase which is very specifically involved in the degradation of peptide substrates having an Asp or Glu residue in the N-terminal position.

Of the biologically active peptides of the central or peripheral nervous system, the C-terminal octapeptide of cholecystokinine CCK_8 and angiotensin II are precisely physiological substrates of aminopeptidase A.

CCK_8 interacts with two types of receptors, the CCK-A sites located chiefly at the periphery and the CCK-B receptors preferably located in the central nervous system. At the peripheral level, CCK_8 stimulates the contractions of the bile duct and ileum, increases intestinal and gastric motility, promotes the secretion of pancreatic enzymes. In the central nervous system, CCK_8 regulates alimentary behaviour, promotes the release of hypophyseal hormones and leads to behavioural changes linked with anxiety.

Consequently, the use of CCK_8 degradation inhibitors constitutes an effective method of intervening in these different physiological processes and thereby changing them.

As for angiotensin II and its metabolite, angiotensin III, they are part of the angiotensinergic cascade, interact with the receptors ATI and ATII and intervene in a different way in the central and peripheral control of blood pressure and the release of hypophyseal hormones such as vasopressin.

Consequently, in this particular case, it is possible to modulate arterial pressure and the release of vasopressin by inhibiting the degradation of

angiotensin II into angiotensin III. This would be a therapeutic method of treating essential and secondary arterial hypertension, cardiac and renal failure, disorders of hydrodynamic homeostasis, myocardial infarction and proteinuria in diabetics.

APA inhibitors have already been proposed in the literature. They correspond more particularly to the following general formula A:

$$H_2N\text{-}CH(R_A)CH_2SH \qquad A$$

wherein R_A preferably denotes an aliphatic chain substituted by a negatively charged group (Wilk *and al.* (1990) *Neuropeptides* 16, 163-168 ; Chauvel *and al.* (1994) *J. Med. Chem.* 37, 1339-1346, *J. Med. Chem.* 37, 2950-2956).

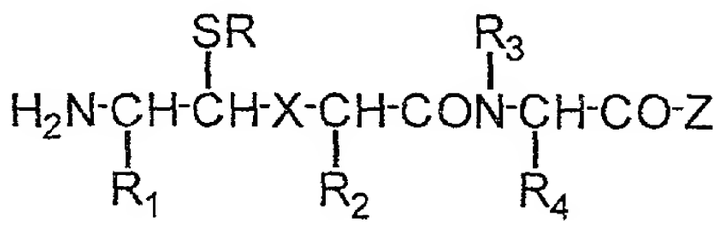
However this family of compounds has the major disadvantage of having no selectivity with regard to aminopeptidase A.

Parallel to the inhibition of aminopeptidase A, these compounds also inhibit aminopeptidase N. Thus their Ki are, at best, of the order of 10^{-7} M on APA and their selectivity factor with regard to APN is relatively low (less than 100).

It is clear that this non-selective behaviour constitutes a handicap from the therapeutic point of view.

The present invention sets out precisely to propose a new family of compounds which advantageously has a selective nature with regard to APA.

More precisely, the present invention relates to a compound of general formula I :



I

wherein :

- R_1 denotes an alkyl, alkenyl or alkynyl chain, or a cycloalkyl, or (cycloalkyl)alkyl group substituted by at least one

– -COOH group, optionally esterified by an alkyl group comprising 2 to 12 carbon atoms,

– SO_3H group, optionally protected by a pentyl group,

– PO_3H_2 group, optionally substituted by a ($-CH_2CH_2SCOR_5$) group, with R_5 representing a C_1-C_4 alkyl group, a phenyl or benzyl group, or

– tetrazolyl group.

• R_2 denotes an alkyl chain, or an aryl, arylalkyl, cycloalkyl, (cycloalkyl)alkyl, (heteroaryl)alkyl group which may or may not be substituted by at least one OH, OR, SR', NH_2, NHR', guanidinyl, COOH or $CONH_2$ group, or a halogen atom selected from among F, Cl, Br or I with R' representing a straight-chain or branched C_{1-4} alkyl group.

• R_3 denotes a hydrogen atom or a methyl group,

• R_4 denotes

– an alkyl chain, an aryl, arylalkyl, cycloalkyl, (cycloalkyl)alkyl, (heteroaryl)alkyl, heterocycloalkyl or (heterocycloalkyl)alkyl group substituted by at least one $CONH_2$, SO_3H, SO_2NH_2, PO_3H_2 or tetrazolyl group, with the groups SO_3H and PO_3H_2 optionally protected as described above,

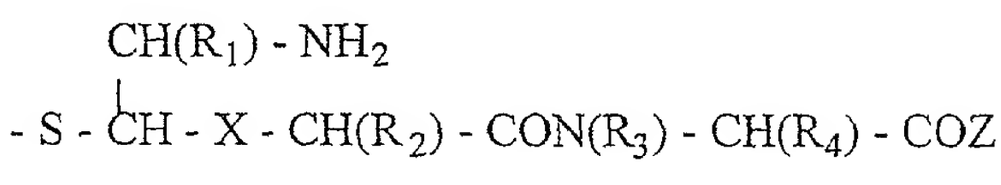
– a C_{2-6} alkyl chain, an aryl, arylalkyl, cycloalkyl, (cycloalkyl)alkyl, (heteroaryl)alkyl, heterocycloalkyl or (heterocycloalkyl)alkyl group substituted by at least one CO_2H group optionally protected as described above, or

• R_3 and R_4 may together constitute a 5- or 6-membered heterocyclic compound, substituted by at least one CO_2H, $CONH_2$, SO_3H, SO_2NH_2 or PO_3H_2 group with the groups CO_2H, SO_3H and PO_3H_2 optionally protected as described above,

• X denotes a group CONH or CH_2NH,

• Z denotes a group OH, OCH_2-C_6H_5 or NR"R"' wherein R" and R"' may denote independently of one another a hydrogen atom or an alkyl, aryl or arylalkyl group, where R" and R"' may constitute, together with the nitrogen atom, a 5- or 6-membered heterocycle possibly having a second heteroatom selected from among O, S and N,

- R denotes a hydrogen atom or a group of formula II



II

corresponding to the symmetric disulphide of the inhibitor wherein R_1, R_2, R_3, R_4, X and Z are defined as hereinbefore,

and the derivatives thereof.

The definitions of the various groups proposed in general formula I of the compounds claimed are given in the list which follows. These definitions apply to the terms as used throughout this text (unless they are restricted to precise examples) both individually and as part of a wider group.

Thus , within the scope of the invention, unless stated to the contrary :

- Alkyl denotes a straight-chain or branched saturated aliphatic chain having 1 to 6 carbons.
- Alkenyl denotes a straight-chain or branched aliphatic chain with 2 to 6 carbons having a double bond.
- Alkynyl denotes a C_{2-6} aliphatic chain having a triple bond.
- Cycloalkyl denotes a saturated C_{3-6} hydrocarbon ring.
- Aryl denotes an aromatic C_6 ring which may or may not be fused and/or may or may not be substituted by on or two other aromatic C_6 rings.
- Heteroaryl denotes an aromatic 5- or 6-membered heterocycle containing 1 or 2 heteroatoms selected from among N, S, O, optionally fused or substituted by an aromatic C_6 ring.
- Heterocycloalkyl denotes a saturated 5- or 6-membered heterocycle containing 1 or 2 heteroatoms selected from N, S, O optionally fused or substituted by an aromatic C6 ring.

For the purposes of the present invention, the term "derivatives" refers particularly to the addition salts of the compounds of general formula (I) obtained with pharmacologically acceptable organic or inorganic acids. These may be for example salts such as the hydrochloride, hydrobromide, sulphate,

nitrate, borate, phosphate, methane sulphonate, acetate, fumarate, succinate, ascorbate, oxalate, lactate, pyruvate, citrate, tartrate, maleate, malonate, benzoate, diaminobenzene sulphonate, cromoglycate, benzene sulphonate, cyclohexane sulphonate, toluene sulphonate, dipropyl acetate, glucose-1 phosphate, palmoate and palmitate.

Of these derivatives, mention may also be made of the dimers of compounds of general formula I, consisting of two molecules of compounds of general formula I, identical or different, coupled to each other at their respective sulphur atoms. In this particular case, R denotes the group of formula II identified hereinbefore.

Similarly, the present invention also includes the different enantiomeric forms of the compounds claimed.

In fact, the compounds of formula (I) which have a number of asymmetric carbons are present in the form of either racemic or diastereomeric mixtures or in the form of the pure stereoisomers.

The optically pure compounds may be isolated by enantioselective syntheses or resolution by chiral amines. In the case of methods of preparation which produce mixtures of stereoisomers, separation by semi-preparative column HPLC (Kromasil C_{18}, 20x250 mm, CH_3CN-H_2O) is carried out, allowing each stereoisomer to undergo separate biochemical and pharmacological investigation.

Of these stereoisomers, those having an absolute (S) or (R) configuration on the carbon carrying the group R_1, an (S) or (R) configuration on the carbon carrying the -SR function, and an (S) configuration on the carbons carrying the groups R_2 and R_4 are preferred .

According to a preferred embodiment of the invention, the compounds claimed correspond to general formula II wherein

- R_4 denotes an alkyl chain, an aryl, arylalkyl, cycloalkyl, (cycloalkyl)alkyl, (heteroaryl)alkyl, heterocycloalkyl or (heterocycloalkyl)alkyl group substituted by at least one $CONH_2$, SO_3H, SO_2NH_2, PO_3H_2 or tetrazolyl group, with the groups SO_3H and PO_3H_2 optionally being protected, or

• R_4 constitutes with R_3 a 5- or 6-membered heterocyclic compound, substituted by at least one CO_2H, $CONH_2$, SO_3H, SO_2NH_2 or PO_3H_2 group with the groups CO_2H, SO_3H and PO_3H_2 optionally protected as described above.

Of particular interest are the compounds claimed wherein R_4 and R_3 together constitute a 5- or 6-membered heterocyclic compound substituted by at least one CO_2H, $CONH_2$, SO_3H, SO_2NH_2 or PO_3H_2 group with the groups CO_2H, SO_3H and PO_3H_2 optionally being protected as described above.

According to a preferred embodiment of the invention, the compounds claimed correspond to general formula II wherein X denotes a CONH function and more preferably R denotes a hydrogen atom.

More preferably, R_2 denotes an alkyl or arylalkyl chain which is optionally substituted, preferably by a hydroxyl group.

The following may be mentioned as examples of compounds according to the present invention :

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Tyr-L.Sal-OH ;

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Tyr-L.hSal-OH ;

N-[[(2S,3R)- and (2R,3R)-3-amino-5-carboxy-2-mercapto] pentanoyl]-L.Ile-L.(3R)(3-COOH)Pro-OH ;

N-[[(2S,3R)- and (2R,3R)-3-amino-5-phosphonate-2-mercapto] pentanoyl]-L.Ile-L.Glu-OH ;

the N-[[2S,3R) and (2R,3R), 3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.Sal-OH ;

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.(3R)(3-COOH)Pro-OH ;

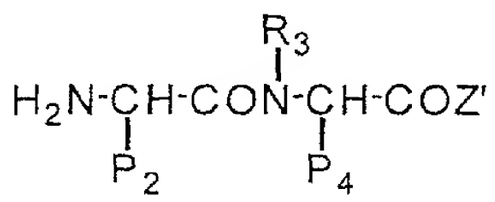
N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.(3S)(3-COOH)Pro-OH ; and

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.Glu-NH_2.

In the above compounds, the abbreviations Sal and hSal denote the sulphoalanine and homosulphoalanine groups, respectively.

The compounds claimed may be obtained by various methods of synthesis depending on the definition of the group X.

The present invention also relates to a process which may be used to prepare the compounds of general formula (I) wherein X denotes a CONH group comprising at least coupling an ester dipeptide of general formula III



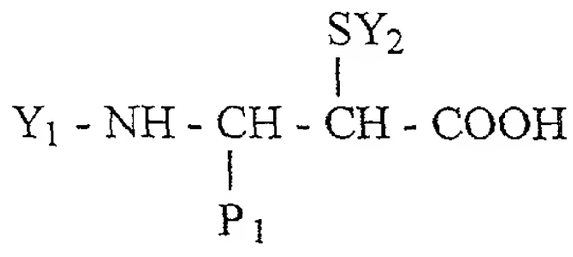
III

wherein

- P_2 and P_4 correspond to protected forms of R_2 and R_4,
- R_3 is as hereinbefore defined and
- Z' denotes a group $OC(CH_3)_3$, OCH_2-C_6H_5 or NR"R'"

wherein R" and R'" independently of one another may denote a hydrogen atom or an alkyl, aryl or arylalkyl group, while R" and R'" may constitute, together with the nitrogen atom, a 5- or 6-membered heterocycle possibly having a second heteroatom selected from among O, S and N,

with a compound of general formula IV

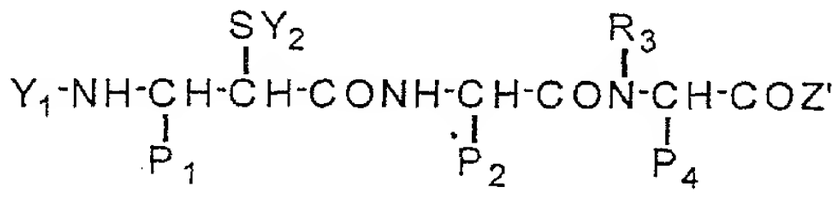


IV

wherein :

- Y_1 denotes a protecting group
- Y_2 denotes a protecting group and
- P_1 denotes a protected form of R_1,

under conditions suitable to produce compound V



V

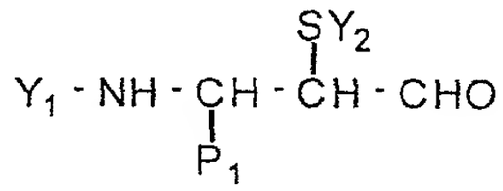
and, by deprotecting it, obtain said compound of general formula I.

The coupling reaction is carried out in conventional manner in an organic solvent, in the presence of a coupling agent and a tertiary amine, at a temperature of the order of 20°C.

Generally, the coupling of the acid of formula (IV) with the amine of general formula (III) is carried out by working in an organic solvent such as dichloromethane or dimethylformamide, using as coupling reagent a BOP-type compound [benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate], HATU [O-(7-azabenzotriazol-1-yl)1,1,3,3-tetramethyluronium hexafluorophosphate], or TFFH [tetramethylfluoro-formamidinium hexafluorophosphate] at a temperature of the reaction mixture of about 20°C and in the presence of a tertiary amine such as diisopropylethylamine.

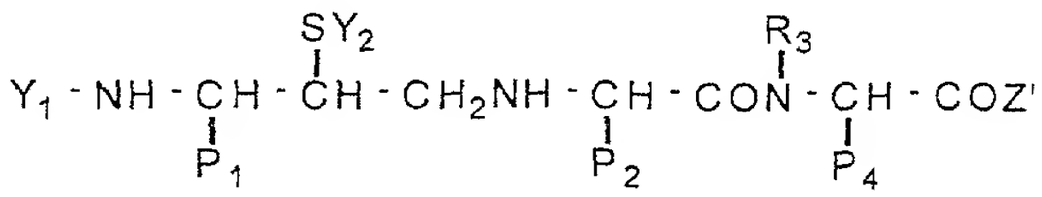
The coupling conditions preferably use as reagent BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) in the presence of diisopropylethylamine in CH_2Cl_2.

The compounds of general formula (I) wherein X denotes a CH_2NH group may be obtained by a process comprising at least condensing a compound of general formula III as defined hereinbefore and a compound of general formula VI,



VI

wherein Y_1, Y_2 and P_1 are as hereinbefore defined, then reducing the intermediate thus formed to produce the compound of general formula VII



VII

and, after deprotecting it, obtain the compound of general formula I.

The dipeptide ester of general formula III has two asymmetric carbons. According to a preferred embodiment of the invention, the S configuration of each amino acid is favoured.

As for the compound of general formula IV, it has two asymmetric carbons C_2 and C_3. The stereochemistry of C_3 is defined by that of the amino acid used at the start of the synthesis of said compound IV according to one of the two synthesis diagrams defined hereinafter.

The intermediate may be reduced by any conventional method. Generally, it is reduced *in situ* by adding a sufficient quantity of an organic reducing agent such as a borohydride and more preferably sodium borohydride or cyanoborohydride.

As for the reactions of protection and deprotection these are within the capabilities of those skilled in the art.

As examples of the protecting groups which may be used within the scope of the present invention, a t. butyloxycarbonyl (t. Boc), benzyloxycarbonyl (Cbz) or fluorenyloxycarbonyl (Fmoc) group is particularly preferred for Y_1, and a p. methoxybenzyl or dimethoxybenzyl group is particularly preferred for Y_2.

Of course, these protecting groups are selected by considering the nature of the reaction to which the corresponding compounds are to be subjected and so as to effectively protect the functional groups in question during this reaction.

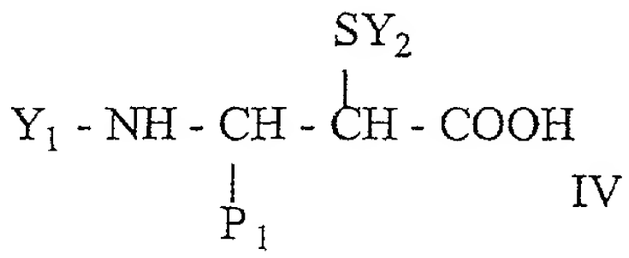
The reactions of deprotection are, of course, selected as a function of the nature of the protecting groups.

Thus, the compounds V and VII wherein Y_1, Y_2, P_1, P_2 and P_4 are preferably as hereinbefore defined are generally deprotected by treating with a mixture of TFA/anisol or BBr_3 in CH_2Cl_2 or HBr in acetic acid, or HF to produce compounds I (with X = CONH and X = CH_2NH, respectively).

However, when P_1 and/or P_4 contain a protected sulphonate group $SO_3CH_2t.Bu$, an additional step of deprotection by heating with tetramethylammonium chloride in DMF is necessary to produce the compounds

I having groups R_1 and/or R_3 which have a free sulphonate, except in the case of deprotection by HF.

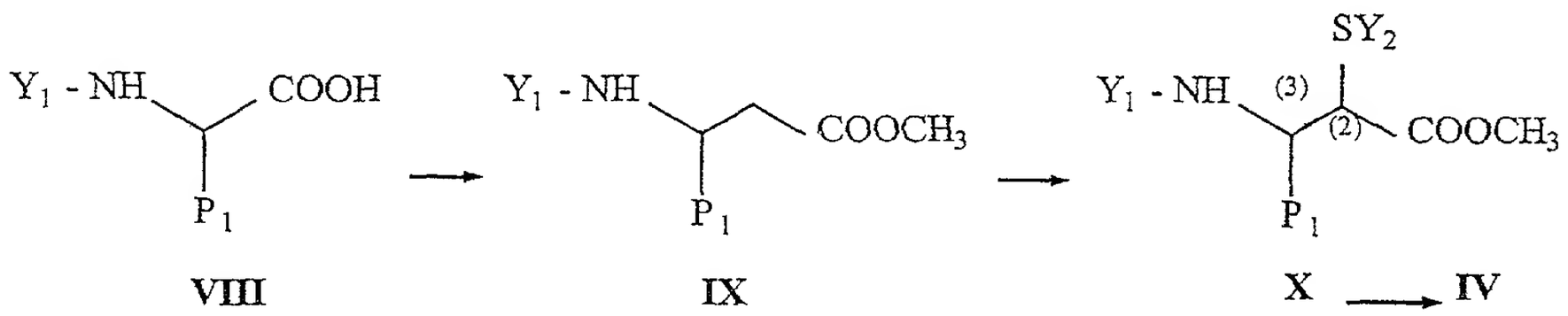
As for the compounds of general formula IV



IV

they may be obtained by two distinct methods of synthesis.

The first method of synthesis is illustrated by the following reaction plan.

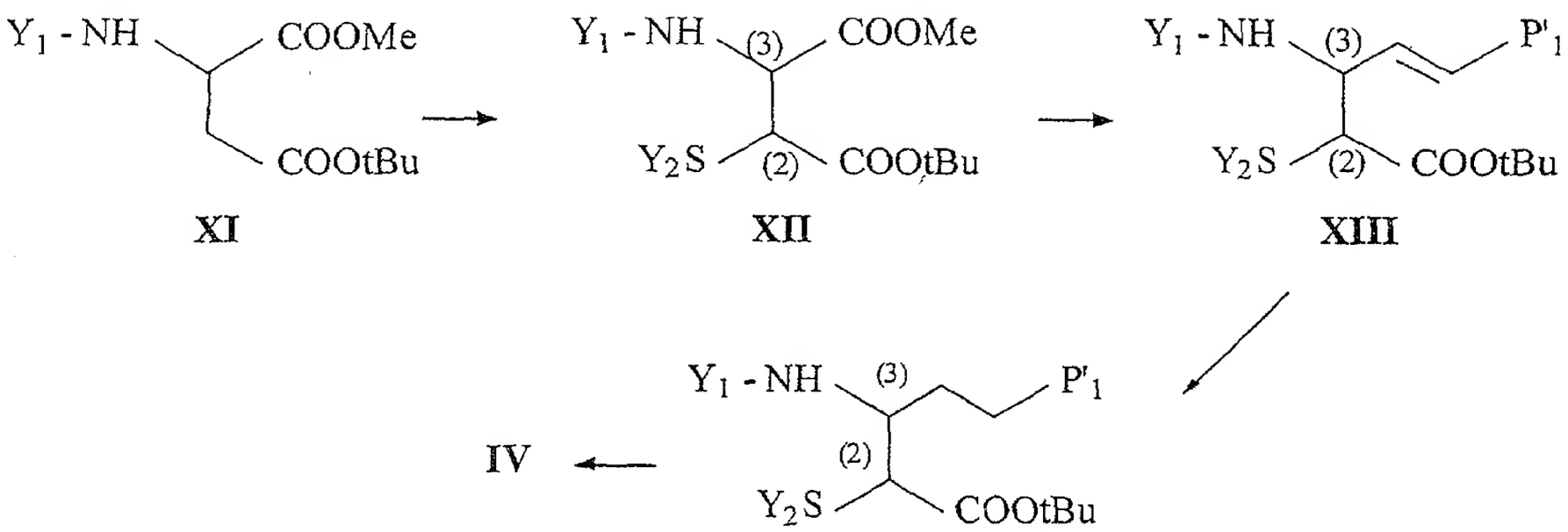


VIII IX X → IV

An α-amino acid protected on the N-terminal VIII is homologated by a method well known in the art into the ß-amino ester IX. A reaction of sulphenylation by the reagents described in Shibata and al (Synlett. (1996)519-520; Tetrahedron 52(1996)12839-12852) or in Bischoff and al. (J. Org. Chem. 62(1997)4848-4850) in the presence of LDA or LiHMDS then leads to the derivative X which, after alkaline hydrolysis of the methyl ester, produces the compound IV.

The N-protected α-amino acids VIII are commercially available or may be prepared under enantioselective conditions using the method of Oppolzer (*Tetrahedron Lett.* 30(1989)6009-6010).

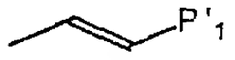
A second method of synthesis was used, illustrated by the following reaction plan.



This second method of synthesis has the advantage of being diastereoselective. It comprises steps consisting of :

– sulphenylating the protected diester N of formula XI of aspartic acid of the given configuration 2 into a compound XII whose configurations in positions 2 and 3 are identical but reversed compared with the configuration of the compound of formula XI,

– reducing the ester α group into aldehyde,

– condensing it *in situ* with an organic reagent so as to obtain a compound of general formula XIII of the same configuration and carrying a group P'_1 which is such that  is a precursor of the group P_1,

– reducing said compound of formula XIII into a compound XIV in the form of 2 diastereoisomers and

– hydrolysing it in an acid medium to obtain the expected compound of formula IV.

The following may be proposed, in particular, as an example of this type of method of synthesis:

The N protected diester XI of aspartic acid of S configuration, for example, is sulphenylated as described in the previous method into compound

XI of the 2R,3R configuration. The α ester group is reduced by dibal (diisobutyl aluminium hydride) into aluminoxyacetal, then condensed in situ with a dialkylphosphonate or a phosphonium ylide, thus leading to the unsaturated compound XII of the same configuration. The reduction of XII by a copper hydride $[(Ph_3P)CuH]_6$ leads to XIII, predominantly of the same configuration (d.e. 85%). The reduction of the double bond by $NaBH_4$, by boron derivatives such as diborane in acetic acid, or cyclohexane in the presence of palladium leads to XIII in the form of 2 diastereoisomers. By acid hydrolysis, compound IV is obtained.

As stated previously, the compound IV has two asymmetric carbons, C_2 and C_3. The stereochemistry of C_3 is defined by that of the amino acid used at the start of synthesis (diagram 1 or diagram 2) as it is not modified during the different steps.

The reaction of sulphenylation is diastereoselective and is carried out in the anti position.

During the steps of reduction in a basic medium (XIII → XIV) epimerisation on the carbon C_2 at the bottom of the sulphur leads to the missing stereoisomers which are separated by column chromatography after coupling of the dipeptide or by HPLC after coupling and partial deprotection.

In this way the four stereoisomers of the compound of general formula IV are obtained. According to a preferred embodiment of the invention, the formation of the 2R, 3R isomer is favoured.

The compounds claimed have proved capable of significantly and selectively inhibiting aminopeptidase A in relation to aminopeptidase N.

This selectivity factor is generally greater than 10^2 and more preferably 10^3.

Consequently, the present invention also relates to the use of the compounds claimed as selective inhibitors of aminopeptidase A.

In this capacity, the compounds claimed may be used in therapy in all pathological processes induced by physiological substrates of aminopeptidase A such as the C-terminal octapeptide of cholecystokinine CCK8 and angiotensin II , the physiological roles of which were discussed earlier.

They may thus be used to reduce food intake, modulate anxiety states or panic attacks or treat essential and secondary arterial hypertension, cardiac and renal failure, disorders of hydrodynamic homeostasis, myocardial infarction and proteinuria in diabetics.

For these purposes, the compounds claimed and the derivatives thereof may be used to prepare corresponding pharmaceutical compositions.

More particularly, the present invention relates, in another aspect, to a pharmaceutical composition containing as active ingredient at least one compound of general formula (I) or a derivative thereof.

Of course, this compound may be combined with at least one pharmaceutically acceptable carrier.

It is also possible to introduce two or more compounds of general formula I together into a single pharmaceutical composition.

These pharmaceutical compositions may be administered by oral, parenteral, sublingual, transdermal or topical route.

For administration by oral or sublingual route, plain or coated tablets, gelatine capsules, granules, optionally with delayed release, drops or liposomes may be used, in particular. For administration by intravenous, subcutaneous or intramuscular route, sterile or sterilisable solutions may be used, in particular, for venous perfusion, whereas conventional patches may be produced for administration by transdermal route.

The pharmaceutical compositions according to the present invention may be prepared by conventional methods well known in the field of pharmaceutical technology.

The active ingredient may be incorporated in the excipients normally used in these pharmaceutical compositions, such as talc, gum arabic, lactose, starch, magnesium stearate, aqueous or non-aqueous carriers, animal or vegetable fats, paraffin derivatives, glycols, various wetting, dispersing or emulsifying agents, preservatives, etc.

The quantity of active ingredient to be administered per day will depend of course on the nature of the therapeutic indication, the seriousness of the complaint to be treated as well as the patient's body weight and the route of administration.

For systemic administration, the overall dose in humans generally varies between 1 and 100 mg per day, for example 2 to 50 mg, and more appropriately 3 to 40 mg per day.

Single-dose forms for systemic administration will generally contain from 3 to 50 mg (i.e. 3, 5, 10, 20, 30, 40, and 50 mg de product). These single doses will normally be given one or more times a day, preferably one to three times a day.

For topical administration, the pharmaceutical compositions generally contain 0.0001 to 1 % of active ingredient and preferably 0.01 to 5 %.

The instant invention relates also to a method for the prevention or treatment of anxiety states or panic attacks, essential and secondary arterial hypertension, cardiac and renal failure, disorders of hydrodynamic homeostasis, myocardial infarct and proteinuria in diabetics, comprising administering to a patient in need of such treatment a therapeutically efficient amount of a compound of general formula (I).

The compounds described according to the invention may also be used in non-therapeutic applications, particularly in systems for diagnosing and measuring the expression of aminopeptidase A.

It is also claimed a method of diagnosis of anxiety states or panic attacks, essential and secondary arterial hypertension, cardiac and renal failure, disorders of hydrodynamic homeostasis, myocardial infarct and proteinuria in diabetics, wherein the aminopeptidase A is evaluated, in a biological sample of a patient to be tested, by using a claimed compound and is compared to the level present in normal subjects.

The present invention also relates to a diagnostic system for detecting and titrating aminopeptidase A, characterised in that it contains at least one compound of general formula I.

The following Examples, provided in a non-restrictive capacity, will demonstrate other advantages of the present invention.

EXAMPLE – 1 -

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Tyr-L.Sal-OH

STEP 1.

4-methyl and tert.butyl (2R,3R), 3-benzyloxycarbonylamino- 2-(4-methoxybenzyl - sulphanyl)succinate.

Under argon, a 2.5 M solution of n-butyl lithium in n-hexane (56 ml ; 140 mmol) is added dropwise at 0°C to a solution of 1,1,1,3,3,3-hexamethyldisilazane (36 ml ; 171 mmol) in dry tetrahydrofuran (375 ml) and the medium is shaken for ~15 min at 0°C. The solution is cooled to –78°C and the compound Z-L.Asp(OtBu)-OMe (20.7 g ; 61 mmol) in dry THF (255 ml) is added dropwise. After stirring the reaction mixture for 2 h between –40°C and –30°C, the medium is cooled to –78°C and solid 4-methoxybenzyl and 2,4-dinitrophenyl disulphide (30 g ; 85.4 mmol) is added. After 1 h at –78°C, the medium is hydrolysed with 1N HCl (200 ml) and the product is extracted with 500 ml of Et_2O. The organic phase is washed with 100 ml of citric acid, 100 ml of H_2O, 100 ml of saturated NaCl, dried over Na_2SO_4 and evaporated to dryness. The residue is taken up in cold Et_2O and the excess precipitate of sulphenylating agent is filtered. After evaporation to dryness the filtrate is purified by flash chromatography on silica gel (eluant cHex, AE, CH_2Cl_2 8 : 1 : 1, R_f = 0,19) giving yellow crystals, 20 g (68 %) (ee : 95/5). HPLC C_{18} Kromasil (5 µ, 100 Å) isocratic CH_3CN/H_2O (TFA) 75 : 25, t_R = 9,9 min, P_f = 71.6-73.3°C.

STEP 2.

tert.butyl (2R,3R)- 3-benzyloxycarbonylamino- 2-(4-methoxybenzyl sulphanyl)- 5-(2,2-dimethylpropanoxysulphonyl)- pent-4-enoate.

A 2.5 M solution of n-butyl lithium in hexane (3.5 ml ; 8.6 mmol) is added dropwise to a solution of neopentyl diethylphosphonomethane sulphonate (2.5 g ; 8.2 mmol) in dry THF (37 ml) at -78°C under argon. The mixture is stirred for 30 min at -78°C, then a solution of the compound of step 1 (2 g ; 4.1 mmol) in dry THF (4 ml) is added, followed, dropwise, by a 1.5 M solution of Dibal-H in toluene (5.3 ml ; 8 mmol). After 4 hours' stirring at -78°C and 1 hour's stirring at

ambient temperature, the reaction mixture is hydrolysed with H_2O (8 ml) and 2N HCl (8 ml). The aqueous phase is then extracted with 160 ml of EA. The organic phase is washed with saturated NaCl, dried over Na_2SO_4 and the solvent is evaporated to dryness. The pure product is obtained after flash chromatography (eluant cHex, EA, CH_2Cl_2 7 : 1.5 : 1.5, R_f = 0,39) in the form of a yellow oil, 1.6 g (68 %). HPLC C_{18} Kromasil (5 µ, 100 Å) isocratic CH_3CN/H_2O (TFA) 75 : 25 t_R = 17,1 min. SM (Electrospray) : 629,8 = MNa^+.

STEP 3.

• *Procedure 1.*

tert.butyl (2RS,3R), 3-benzyloxycarbonylamino-2-(4-methoxybenzyl sulphanyl)- 5-(2,2-dimethylpropanoxysulphonyl)pentanoate.

The ethylene compound obtained in step 2 (10 g ; 16.4 mmol) in EtOH (164 ml) is reduced by $NaBH_4$ (996 mg ; 26.3 mmol) for 3 h at ambient temperature. After a conventional treatment, the product is obtained in the form of an orange oil, 10 g (99 %). R_f (cHex, CH_2Cl_2, EA 6 : 2 : 2) = 0.50. It has total epimerisation at the C_2. HPLC C_{18} Kromasil (5 µ, 100 Å) isocratic CH_3CN/H_2O (TFA) 75 : 25 t_R = 15.9 min and 16.6 min.

• *Procedure 2.*

tert.butyl (2R,3R), 3-benzyloxycarbonylamino- 2-(4-methoxybenzyl sulphanyl)- 5-(2,2-dimethylpropanoxysulphonyl)pentanoate.

A solution of the previous ethylene compound (100 mg ; 0.16 mmol) in benzene (2.5 ml) is degassed under argon for 15 minutes. To this solution are added, successively, at ambient temperature, 60 µl (3.3 mmol) of water, then 120 mg (0.06 mmol) of $[(Ph_3P)CuH]_6$. The reaction medium is stirred for 3 hours at ambient temperature then hydrolysed with a solution of NH_4Cl, and extracted with EA. The organic phase is washed with saturated NaCl, dried over Na_2SO_4 and evaporated to dryness, giving the product (80 mg ; 80 %) in the form of a yellow oil having an enantiomeric excess of 85 : 15. HPLC C_{18} Kromasil (5 µ, 100 Å) isocratic CH_3CN/H_2O (TFA) 75 : 25 t_R = 16.6 min.

STEP 4.

(2RS, 3R), 3-benzyloxycarbonylamino- 2-(4-methoxybenzyl sulphanyl)-5-(2,2-dimethylpropanoxysulphonyl) pentanoic acid.

A solution of the compound of step 3 (10 g ; 16.4 mmol) in CH_2Cl_2 (13 ml) is treated with anisol (630 µl ; 5 % vol.) and TFA (12.6 ml ; 164 mmol) and after 3 hours' stirring at ambient temperature leads to the expected acid, which is chromatographed (silica gel, flash, eluant cHex, Et_2O, HCOOH 4 : 6 : 0.1, R_f = 0.40) giving a red foam, 6.1 g (69 %). HPLC C_{18} Kromasil (5 µ, 100 Å) isocratic CH_3CN/H_2O (TFA) 75 : 25 t_R = 5.6 min.

STEP 5.

N-[(2S,3R) and (2R,3R)- 3-benzyloxycarbonylamino-5-[2,2-dimethylpropanoxy-sulphonyl]- 2-[4-methoxybenzylsulphanyl]pentanoyl]-L.Tyr(t.Bu)-L.Sal(OCH_2tBu)OtBu

The acid is coupled in the solid phase with the dipeptide H-Tyr(tBu)-Sal(OCH_2tBu) grafted onto a Wang-type resin using the coupling agent BOP and diisopropylethylamine.

STEP 6.

N-[(2R,3R) and (2S,3R)- 3-benzyloxycarbonylamino-5-[2,2-dimethylpropanoxy - sulphonyl]-2-[4-methoxybenzylsulphanyl]pentanoyl]-L.Tyr-L.Sal-OH.

Peptidyl resin in CH_2Cl_2 (2 ml) is treated at 0°C with 60 µl of anisol (5 % vol.) and trifluoroacetic acid (1.2 ml ; 15 mmol) and the reaction mixture is stirred for 3.5 hours at ambient temperature. After evaporation to dryness, the residue is taken up in cold Et_2O and the precipitate obtained is triturated in the same solvent. The product is recovered, after decanting the ethereal phase, in the form of a white powder.

STEP 7.

N-[[(2RS,3R), 3-amino-2-mercapto-5-sulphonate]pentanoyl]-L.Tyr-L.Sal-OH.

The pseudo-tripeptide of step 6 (0.43 mmol) is treated with 500 µl of meta-cresol distilled beforehand, then, at -78°C, with 10 ml of liquid hydrogen fluoride. After 1 hour's stirring at 0°C, the HF is evaporated in vacuo. The residue is

taken up in 2 x 1 ml of TFA and the product is obtained by precipitation in cold Et_2O/n-hexane and centrifugation. The compound is finally purified by semi-preparative HPLC with C_{18} Kromasil (10 µ, 100 Å) on a CH_3CN/H_2O (TFA) gradient from 10 to 60%, t_R = 4.1 min, to yield a white powder (20 mg). SM (Electrospray) : 544.6 = MH^+, 566.6 = MNa^+.

EXAMPLE 2-

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Tyr-L.hSal-OH

In accordance with the process used in Example 1 with the dipeptide H-Tyr(tBu)-hSal(OCH_2tBu) grafted onto a Wang resin, the two stereoisomers separated are obtained in the form of 2 white powders (14 and 17 mg). HPLC C_8 Kromasil (5 µ, 100 Å) gradient CH_3CN/H_2O (TFA) from 10 to 60 %, t_R = 2.8 min and 3.4 min. SM (Electrospray) : 557.9 = MH^+, 579.9 = MNa^+.

EXAMPLE 3 -

N-[[(2S,3R)- and (2R,3R)-3-amino-5-carboxy-2-mercapto] pentanoyl]-L.Ile-L.(3R)(3-COOH)Pro-OH

This compound is obtained using the procedure described for Example 1, using Z-L-Asp (OtBu)OMe in step 1, Ile-(3R)(3-CO_2CH_2Ph)ProOCH_2Ph dipeptide in step 5 and omitting step 6.

3A : [[(2S,3R)-3-amino-5-carboxy-2-mercapto]pentanoyl]-L.Ile-L.(3R)(3-COOH)Pro-OH

White powder (5 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 8,5 min. SM (Electrospray) : 448,6 = MH^+.

3B : [[(2R,3R), 3-amino-5-carboxy-2-mercapto]-pentanoyl]-L.Ile-L.(3R)(3-COOH)Pro-OH

White powder (10 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 6.0 min. SM (Electrospray) : 448.0 = MH^+.

EXAMPLE – 4 -

N-[[(2S,3R)- and (2R,3R)-3-amino-5-phosphonate-2-mercapto] pentanoyl]-L.Ile-L.Glu-OH

This compound is obtained using the procedure described for Example 1, using tetrabenzyl diphosphoryl methylene in step 2 and Ile-Glu(OtBu)OtBu dipeptide in step 5.

4A : [[(2S,3R)-3-amino-5-phosphonate-2-mercapto]-pentanoyl]-L.Ile-L.Glu-OH

White powder (23 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 6.97 min. SM (Electrospray) : 472.4 = MH^+.

4B : [[(2R,3R)-3-amino-5-phosphonate-2-mercapto]-pentanoyl]-L.Ile-L.Glu-OH

White powder (33 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 4.6 min. SM (Electrospray) : 472.5 = MH^+.

EXAMPLE – 5 -

N-[[2S,3R) and (2R,3R), 3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.Sal-OH

This compound is obtained using the procedure described for Example 1, using the dipeptide Ile-Sal(CH_2tBu)OH in step 5.

5A : [[(2S,3R)- 3-amino-2-mercapto-5-sulphonate]-pentanoyl]-L.Ile-L.Sal-OH

White powder (4 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 4.1 min. SM : 493.9 = MH^+.

5B : [[(2R,3R), 3-amino-2-mercapto-5-sulphonate]-pentanoyl]- L.Ile-L.Sal-OH

White powder (13 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 2.4 min.

EXAMPLE 6 -

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.(3R)(3-COOH)Pro-OH

This compound is obtained using the procedure described for Example 1, using the dipeptide Ile-(3R)(3-CO_2CH_2Ph)Pro OCH_2Ph in step 5 and omitting step 6.

6A : [[(2S,3R), 3-amino, 2-mercapto-5, sulphonate]-pentanoyl]- L.Ile-L.(3R)(3-COOH)Pro-OH

White powder (19 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 9.9 min. SM : 484.1 = MH^+, 506.1 =MNa^+.

6B : [[(2R,3R), 3-amino-2-mercapto-5-sulphonate]-pentanoyl]- L.Ile-L.(3R)(3-COOH)Pro-OH

White powder (33 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 3.6 min. SM : 484.1 = MH^+.

EXAMPLE 7 -

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.(3S)(3-COOH)Pro-OH

This compound is obtained using the procedure described for Example 1, using the dipeptide Ile-(3S)(3-CO_2CH_2Ph)ProOCH_2Ph in step 5 and omitting step 6.

7A : [[(2S,3R), 3-amino-2-mercapto-5-sulphonate]-pentanoyl]-L.Ile-L.(3S)(3-COOH)Pro-OH

White powder (4 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 14.9 min. SM : 484.5 = MH^+, 522.5 = MK^+.

7B : [[(2R,3R), 3-amino-2-mercapto-5-sulphonate]-pentanoyl]-L.Ile-L.(3S)(3-COOH)Pro-OH

White powder (6 mg). HPLC C_{18} Kromasil (5µ, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R= 4.7 min. SM : 484.5 = MH^+, 522.3 = MK^+.

EXAMPLE – 8 -

N-[[(2S,3R)- and (2R,3R)-3-amino-2-mercapto-5-sulphonate] pentanoyl]-L.Ile-L.Glu-NH_2

By following steps 1 to 7 described in Example 1 but coupling with the dipeptide H-Ile-Glu(OtBu)-NH_2, the two stereoisomers of the desired compound are obtained, in the form of 2 white powders (20 mg and 10 mg). HPLC C_{18} Kromasil (5 µ, 100 Å) isocratic CH_3CN/H_2O (TFA) 15/85, t_R = 3.5 min and 4.9 min. SM (Electrospray) : 470.9 = MH^+.

Benzyl diethyl phosphoryl acetate or benzyl (diethoxy-phosphoryl)-acetate.

This reagent is synthesised according to the procedure described by Jennings and coll. (1992).

Tetrabenzyl diphosphoryl methylene or dibenzyl (dibenzyloxy-phosphoryl-methyl)-phosphonate.

This reagent is synthesised according to the procedure described by Saady and coll. (1995).

EXAMPLE 9 -

IN VITRO STUDY OF THE INHIBITING POWER OF THE SYNTHESISED COMPOUNDS ON AMINOPEPTIDASE A AND AMINOPEPTIDASE N.

a) *In vitro* study of the inhibiting power of the synthesised compounds on aminopeptidase A.

1°) Purification of the enzyme.

Aminopeptidase A is obtained from rabbit kidney as described in "*Neuropeptides 16 (1990)163-168*". The synthetic substrate used is L-glutamyl-ß-naphthylamide (Glu NA, Km = 130 µM). The purified enzyme has a specific activity with regard to Glu NA of 100 $\mu mol.mL^{-1}.li^{-1}$.

2°)Titration method.

The process described by Goldbarg "*Cancer 11 (1958)283-291*" was scaled down for the microtitre plate.

The APA is incubated for 1 h at 37°C in the presence of 200 µM Glu NA and in the presence of variable concentrations of inhibitors in Tris HCl buffer (50 mM),

pH = 7.4, 4 mM of $CaCl_2$ (final volume 100 µl). The reaction is stopped by the addition of 10 µl of 3N HCl. Then 25 µl of $NaNO_2$ (87 mM) are added to each well followed, 3 min later, by 50 µl of ammonium sulphamate (0.13 M). Finally, after 5 min, 25 µl of a 23 mM solution of 1-naphthylethylene diamine are added at 37°C. The absorption at 560 nm is measured and compared with that of a standard calibration range of 2-naphthylamine.

b)) *In vitro* study of the inhibiting power of the synthesised compounds on aminopeptidase N.

1°) Source of the enzyme.

Aminopeptidase N purified from pig's kidney is marketed by Böehringer Mannheim (Meylan, France).

2°) Titration method.

The APN is incubated for 10 min at 37°C in the absence or in the presence of increasing concentrations of inhibitors in a total volume of 100 µl in Tris HCl buffer (50 mM, pH = 7.4. 200 µM of Ala-Na are added and the whole thing is incubated for 30 min at 37°C. The reaction is stopped by the addition of 10 µl of CH_3COO-Na 1M (pH = 4.2). The absorption is measured at 400 nM and compared with that of a standard range of 2-naphthylamine.

	R_1	X	R_2	R_3	R_4	Z	Example	Ki(M) APA	Ki(M) APN
Ex 1	$(CH_2)_2$-SO_3^-	CO	CH_2-p(C_6H_4)-OH	H	CH_2SO_3H	OH	1A+B	$3,7 . 10^{-8}$	$1,0 . 10^{-5}$
Ex 2	$(CH_2)_2$-SO_3^-	CO	CH_2-p(C_6H_4)-OH	H	$(CH_2)_2SO_3H$	OH	2A 2B	$9,3 . 10^{-9}$ $7,3 . 10^{-8}$	$4,6 . 10^{-6}$ $7,5 . 10^{-6}$
Ex 3	$(CH_2)_2$-CO_2^-	CO	$CH(CH_3)CH_2CH_3$	(3R)(3-CO_2H)Pro		OH	3A 3B	$0,72 . 10^{-9}$ $6,5 . 10^{-8}$	$>5 . 10^{-6}$ $>5 . 10^{-6}$
Ex 4	$(CH_2)_2$-P(O)(OH)-O^-	CO	$CH(CH_3)CH_2CH_3$	H	$(CH_2)_2$-CO_2H	OH	4A 4B	$1,4 . 10^{-8}$ $9,5 . 10^{-7}$	$>5 . 10^{-6}$ $>5 . 10^{-6}$
Ex 5	$(CH_2)_2$-SO_3^-	CO	$CH(CH_3)CH_2CH_3$	H	CH_2-SO_3H	OH	5A 5B	$3,6 . 10^{-9}$ $8,3 . 10^{-8}$	$>5 . 10^{-6}$ $>5 . 10^{-6}$
Ex 6	$(CH_2)_2$-SO_3^-	CO	$CH(CH_3)CH_2CH_3$	(3R)(3-CO_2H)Pro		OH	6A 6B	$0,87 . 10^{-9}$ $1,1 . 10^{-7}$	$1,64 . 10^{-5}$ $> 10^{-5}$
Ex 7	$(CH_2)_2$-SO_3^-	CO	$CH(CH_3)CH_2CH_3$	(3S)(3-CO_2H)Pro		OH	7A 7B	$3,8 . 10^{-9}$ $2,4 . 10^{-7}$	$4,9 . 10^{-6}$ $> 10^{-5}$
Ex 8	$(CH_2)_2$-SO_3^-	CO	$CH(CH_3)CH_2CH_3$	H	$(CH_2)_2$-CO_2H	NH_2	8A 8B	$2,5 . 10^{-8}$ $6,3 . 10^{-7}$	$>5 . 10^{-5}$ $>5 . 10^{-5}$